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(71) Applicant: **ARCHER DANIELS MIDLAND
COMPANY**
Decatur, Illinois 62526 (US)

(72) Inventors:
• **Gugger, Eric T.**
Decatur, Illinois 62526 (US)

• **Dueppen, Daniel G.**
Decatur, Illinois 62526 (US)

(74) Representative: **Jones, Michael Raymond et al**
Haseltine Lake & Co.,
Imperial House,
15-19 Kingsway
London WC2B 6UD (GB)

(54) **Production of isoflavone enriched fractions from soy protein extracts**

(57) The temperature sensitive differential of the solubilities of isoflavones is used to separate them by heating an aqueous soy molasses feed stream. The temperature of the feed stream is increased to select an isoflavone. Then the heated feed stream is passed

through an ultrafiltration membrane. The resulting permeate is cooled to crystallize the isoflavone. Or, the permeate may be put through a resin adsorption process in a liquid-chromatography column to separate out the desired isoflavone. Various processes are described for drying and recrystallizing the resulting isoflavone solids.

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Description

The invention relates to a production of isoflavone rich process streams by a treatment of an aqueous alcohol extract of defatted soybean flakes.

Background of the Invention

Isoflavones are a unique class of plant flavonoids that have a limited distribution in the plant kingdom and may be physically described as colorless, crystalline ketones. The most common and important dietary source of these isoflavones are soybeans which contain the following twelve isoflavone isomers: genistein, genistin, 6"-O-malonylgenistin, 6"-O-acetylgenistin; daidzein, daidzin, 6"-O-malonyldaidzin, 6"-O-acetyl genistin; glycitein, glycitin, 6"-O-malonylglycitin, 6"-O-acetylglycitin (Kudou, Agric. Biol. Chem. 1991, 55, 2227-2233). Ninety-seven to ninety-eight percent of the soybean isoflavones are in the glycosylated form.

Traditionally, individuals have been limited in their use of soy foods to increase their levels of dietary isoflavones because the number and variety of soy foods available in the U.S. marketplace is limited.

The isoflavone, genistin, was first isolated from soybean meal in 1931 by Walz (Justus Liebigs Ann. Chem 489, 118) and later confirmed in 1941 by Walter (J. Amer. Chem. Soc. 63, 3273). Patents have described the production of isoflavone enriched soy-protein products (W0 95/10512; W0 95/10529; W0 95/10530), genistin malonate and daidzin malonate (U.S. Patent 5,141,746), pharmaceutical-type compositions containing isoflavones (U.S. Patents 5,424,331; 4,883,788), and isolation and modification of isoflavones from tempeh (U.S. Patents 4,390,559; 4,366,248; 4,366,082; 4,264,509; 4,232,122; 4,157,984). However, the present patent relates to the manufacture of highly enriched isoflavone products containing either a wide-range of soy isoflavones or highly-purified genistin gained from an ethanol extract of defatted soybean flakes.

Since coronary heart disease (CHD) is a leading cause of death, especially in the United States and other industrialized nations, an elevated total and LDL cholesterol levels are important risk factors affecting human health. In humans, soy protein products appear to lower serum total cholesterol levels by an average of about 9.3% and to lower low-density lipoprotein (LDL) cholesterol by an average of about 12.9% when consumed at an average intake level of 47 g soy protein per day (Anderson et al., NEJM, 333:276-282, 1995).

Isoflavones (Phytoestrogens) are implicated as a class of compounds in soy protein products which is at least partly responsible for this cholesterol-lowering effect in animals (Setchell, in McLachlan JA, ed. Estrogens in the Environment II:69-85, 1985). In addition, studies with primates suggest that soy isoflavones may account for up to about 60-70% of the hypocholesterolemic properties of soy protein (Anthony et al., Circulation, 90:Suppl:I-235. (abstract), 1994; Anthony et al., J. Nutr., 125:Suppl 3S:803S-804S. (abstract), 1995; Anthony et al., Circulation, 91:925. (abstract), 1995).

It has also been suggested that isoflavones have an ability to play a role in the prevention of certain cancers. Japanese women who have consumed diets rich in isoflavones appear to have a very low incidence of breast cancer (Adlercreutz et al., J. Nutr. 125:757S-770S, 1995). Soy products have also been shown to decrease mammary tumor formation or to inhibit mammary tumor progression in rat breast cancer models (Barnes et al., Clin. Biol. Res. 347: 239-253; Hawrylewicz et al., J. Nutr. 121:1693-1698, 1991). Genistein has been shown to inhibit protein tyrosine kinase (Akiyama et al., J. Biol. Chem. 262:5592-5595, 1987), to inhibit angiogenesis (Fotsis et al., Proc. Natl. Acad. Sci. USA 90:2690-2694, 1993), and to induce differentiation in several malignant cell lines (Peterson, J. Nutr. 125:784S-789S, 1995), all of which may be important risk factors in cancer development. Genistein and glycitein (Biochanin A) also appear to inhibit the growth of androgen-dependent and independent prostatic cancer cells in vitro (Peterson and Barnes, Prostate 22:335-345, 1993). Genistein may act as an antioxidant (Wei et al., Nutr. Cancer 20: 1-12, 1993).

Summary of the Invention

Accordingly, an object of this invention is to provide a convenient way for individuals to consume isoflavones either as a nutritional supplement or as an ingredient in more traditional types of food.

In light of the potentially positive role of isoflavones and particularly genistein in the prevention of human disease, an object of this invention is to isolate purified forms of genistin, such as the glycone form of genistein, with or without subsequent recrystallization as a further purification step.

Another object is to produce a product which is enriched in the entire range of soy isoflavones in order to provide isoflavone products for human diet supplementation or to enrich food products or food ingredients.

In keeping with an aspect of the invention, selected isoflavones are extracted based on the differentials of the solubilities of isoflavones in aqueous solutions. Alcohol is removed from an extract of defatted soybean flakes by evaporation. Then, the remaining aqueous solution is subjected to ultrafiltration (UF) at an elevated temperature in order to separate soluble isoflavones from other and insoluble materials. The UF permeate containing the soluble

isoflavones may be treated in either one of two ways: 1) the permeate is treated with an adsorptive resin for enabling a recovery of a broad range of isoflavone isoforms while removing soluble sugars and salts; or 2) the permeate cools to promote a crystallization of genistin. Then, genistin is isolated in a highly purified form by either centrifugation or filtration. The genistin may be further purified by a subsequent recrystallization from aqueous alcohol solutions.

Brief Description of Drawings

These and other objects of this invention will become more apparent from the following specification taken with the attached drawings, in which:

- Fig. 1 is a graph showing the solubility of genistin in water vs. temperature;
 Fig. 2 is a graph showing the concentration of isoflavone in a UF permeate vs. temperature; and
 Fig. 3 is a process flow diagram showing the production of the inventive product.

Detailed Description of the Invention

This invention employs methods based on the differential of solubilities of isoflavones in aqueous solutions. Genistin is the least water soluble of the isoflavone glycosides, is insoluble in cold water, and is only slightly soluble in hot water (Fig. 1).

In greater detail, Fig. 1 shows that the solubility of genistin is practically unchanged as the temperature increases from 4°C to 50°C, but that the solubility increases rapidly as the temperature increases from 70° to 90°C. Therefore, if the manufacturing process is to recover genistin, the recovery step should be carried out at the high temperature end of the scale.

All isoflavone glycosides other than genistin have higher solubilities in water and readily pass through an ultrafiltration membrane, along with other water soluble components. By increasing the temperature of the aqueous solution prior to ultrafiltration, genistin and all other isoflavones can be separated from insoluble materials. The isoflavones in the ultrafiltration permeate can be recovered by treating the solution with a resin, washing the resin with water to remove soluble sugars, and eluting the isoflavones with a mixture of ethanol and water.

The starting material for the inventive processes is derived from an aqueous ethanol extract of hexanedefatted soybean flakes. The defatted soybean flakes are extracted with aqueous ethanol (approximately 60-80% ethanol by volume) at temperatures in the range about 44°-63°C or 120-150°F. This aqueous ethanol extract is then subjected to a vacuum distillation in order to remove ethanol. The alcohol-stripped extract is also known as "soy molasses" or "soy solubles."

Then the extract is adjusted within an appropriate temperature range (about 65-95°C) and subjected to ultrafiltration preferably by using a 10,000 molecular weight cut-off (MWCO) membrane. However, the process is not limited to this 10,000 cut-off membrane since any membrane which enables a filtration of the desired isoflavones may be used. The smallest cut-off membrane suitable for the inventive procedures should pass a molecular weight of 532, which provides a sufficient retention of insoluble material and passage of isoflavones.

The effect of temperature on the concentration of two principle isoflavones, daidzin and genistin, in the UF permeate, is shown in Fig. 2. Cooler temperatures result in lower concentrations of genistin in the UF permeate. Daidzin concentrations are much less affected by temperature. To achieve optimal concentrations of isoflavones in the UF permeate, ultrafiltration should be carried out at a temperature above 65°C.

For example, Fig. 2 shows the differential between the concentration of daidzin and genistin in an aqueous solution permeate subjected to ultrafiltration. Ultrafiltration at 24°C produces a high concentration of daidzin and a low concentration of genistin. Therefore, if the manufacturing step is to recover daidzin and reject genistin, perhaps the recovery should be carried out at the relatively low temperature of 24°C, although the exact temperature may be selected on a basis of how much genistin can be located in the permeate. On the other hand, if the manufacturing process is designed to recover both daidzin and genistin, perhaps it would be better to operate at the crossover point of about 78°C. For genistin, recovery should be carried out at a higher temperature.

A flow diagram representing one example of a manufacturing processes is shown in Fig. 3.

In greater detail, Fig. 3 shows at 20 that the preferred starting material is soy molasses which is subjected to ultrafiltration at 22. At 24, the retentate of the ultrafiltration is further processed, recycled, or otherwise used in another process.

If a batch type process is employed, the volume of the UF retentate fraction 24 is reduced during the ultrafiltration process by about one-third to two-thirds of the original alcohol-stripped extract volume, or stated otherwise is up to 12-15% solids. The UF retentate may be diafiltered with about one to three retentate volumes of water, which has been previously adjusted to be within a temperature range of about 65-95°C in order to recover a greater percentage of isoflavones in the permeate.

With or without the diafiltered permeate, the ultrafiltration permeate at 26 contains a variety of isoflavones and is adjusted to an appropriate temperature (45-95°C). Then, it is treated with an adsorptive resin at 28 in either a batch or chromatography column type process, followed by washing the resin with water at 30. Next, the isoflavones are eluted at 32 with aqueous alcohol (20-100% by volume, at 25-85°C) as either a gradient or single percentage process. The resulting material is dried (not shown in Fig. 3), preferably by evaporation, in order to produce a product which is approximately 30% isoflavones on a solids basis. The alcohol which is used at 32 may be ethanol, methanol, or isopropanol. The resin may be, but is not limited to, ethylvinylbenzene-divinylbenzene, styrene-divinylbenzene or polystyrene polymers, and may be either ionic or non-ionic.

Alternatively or in addition, with or without a diafiltered permeate, the ultrafiltration permeate 26 is adjusted to an appropriate temperature (about 4-45°C) in order to promote genistin crystallization at 34. Highly purified genistin crystals are then removed at 36 by a low-speed centrifugation or filtration and are finally washed with cold water (not shown in Fig. 3). The final product is between 70-90% pure genistin, measured on a dry basis. The genistin crystals can be further purified by recrystallization from aqueous alcohol solutions, such as aqueous ethanol, methanol, or isopropanol.

Examples

1) Ultrafiltration of Soy Solubles

Using a stainless steel steam-heated immersion coil, soy solubles (15.26 kg) were heated to a constant temperature of about 80°C. The soy solubles were then passed through a model 92-HFK-131-UYU spiral wound, polysulfone, 10,000 nominal molecular weight cut-off ultrafiltration membrane (Koch Membrane Systems, Inc., St. Charles, IL) by using a parastaltic pump. Back pressure on the exit side of the membrane was adjusted by means of a hand-tightened clamp to provide a permeate flow of 70 mL/minute. Ultrafiltration was continued until 9.4 kg of permeate was collected leaving 4.8 kg of retentate. Isoflavone profiles of the various fractions are shown below:

Sample	Weight (kg)	%Solids	Total Isoflavones (g)	Genistin (g)	Daidzin (g)
Solubles	15.26	8.65	11.45	4.01	4.30
Retentate	4.8	11.5	4.63	1.75	1.67
Permeate	9.4	7.7	6.6	2.29	2.68

2) Diafiltration of UF Retentate

Ultrafiltration retentate (80°C initial temperature) was subjected to ultrafiltration as described in Example 1, except that 4.8 kg of tap water (25°C) was fed into the retentate at a feed rate which is the same as the permeate rate or flux of the permeate that was being produced. The retentate was then further ultrafiltered to a final weight of 1.93 kg. Isoflavone profiles of the various fractions is shown below:

Sample	Weight (kg)	%Solids	Total Isoflavones (g)	Genistin (g)	Daidzin (g)
Retentate	4.8	11.5	4.63	1.75	1.67
Diafilt. Permeate	7.25	4.28	2.12	0.72	0.96
Diafilt. Retentate	1.93	12.26	2.14	0.91	0.58

3) Adsorption and Recovery of Isoflavones From a Resin

A glass liquid-chromatography column (2.54 cm i.d.) was slurry packed in 70% ethanol with Dow XUS 40323 divinylbenzene, ethylvinylbenzene copolymer resin. The resin was cleaned with an additional 500 mL of 70% wt ethanol followed by 0.1% wt NaOH (500 mL) and water (500 mL). The resin was then back-flushed with water until the resin bed volume had expanded by about one half of its originally packed volume in order to partition the resin by size. The final packed volume was 100 mL. Fresh UF permeate (2000 mL or 20 column volumes) at an initial temperature of 60°C was fed through the resin bed at 6 column volumes/hour or 10 mL/minute. The resin bed was washed with 500 mL of water at 10 mL/minute to remove residual sugars and other impurities. Isoflavones were then eluted from the resin with a linear gradient of 20-95% ethanol (500 mL total) at 10 mL/minute. Next, the entire ethanolic isoflavone containing fraction was vacuum dried to obtain a product with the following profile:

Sample	Weight	Total Isoflavones	Genistin	Daidzin
	(g)	(g)	(g)	(g)
Column Product	6.56	2.2	0.92	0.83

4) Precipitation of Genistin From UF Permeate

A 7.69 kg volume of ultrafiltration permeate (85°C initial temperature) was allowed to cool gradually to an ambient temperature (22°C) during a 16 hour period, with constant stirring. The cooled permeate was then centrifuged at 900 x g for 10 minutes in order to pelletize the genistin precipitate. The supernatant was poured off. The white pellet was diluted with water (100 mL) and recentrifuged at 900 x g for 10 minutes in order to remove any residual supernatant. The white pellet was then vacuum dried to produce 1.02 g of a dried powder. The isoflavone composition of the dried powder was as follows:

Sample	Weight	Total Isoflavones	Genistin	Daidzin
	(g)	(g)	(g)	(g)
Genistin Precipitate	1.02	1.00	0.77	0.17

5) Recrystallization of Genistin From UF Permeate Precipitate

A volume of 80% ethanol (50 mL) was slowly added to 1 g of permeate precipitate while refluxing until the precipitate dissolved. Then, the solution was filtered through Whatman 42 filter paper and slowly cooled (22°C) to produce fine yellow-white crystals. The crystals were harvested by centrifugation at 900 x G for 10 minutes. The supernatant was poured off. Next, the crystals were mixed with 50 mL of water (4°C) and recentrifuged to remove any residual supernatant. The water was then poured off and the crystals were vacuum dried to produce a product with the following isoflavone profile:

Sample	Weight	Total Isoflavones	Genistin	Daidzin
	(g)	(g)	(g)	(g)
Genistin Crystals	0.52	0.52	0.45	0.05

Those who are skilled in the art will readily perceive how to modify the invention. Therefore, the appended claims are to be construed to cover all equivalent structures which fall within the true scope and spirit of the invention.

Claims

1. A process for separating isoflavones in an aqueous vegetable starting material, said process comprising the steps of:
 - (a) heating an aqueous vegetable starting material to a constant temperature selected on a basis of an aqueous solubility for a given isoflavone that is to be recovered;
 - (b) passing the heated starting material of step (a) through an ultrafiltration membrane to provide a permeate, the membrane having a cut-off which passes the given isoflavone;
 - (c) adjusting back pressure in a retentate acting on the membrane of step (b) to adjust a flow of said permeate while continuing said ultrafiltration; and
 - (d) collecting said isoflavones from a permeate.
2. The process of claim 1 wherein said starting material is soy molasses.
3. The process of claim 2 wherein step (a) includes the further process steps of: making an aqueous ethanol extract of hexane-defatted soybean flakes at a temperature in the range of about 44°-63°C.
4. The process of claim 2 wherein said ultrafiltration of step (b) is carried out within a temperature range of about

65°-95°C.

5. The process of claim 2 wherein the collection of isoflavones from the permeate of step (d) includes the further steps of:
 - (d1) treating the permeate with an adsorptive resin;
 - (d2) washing the resin of step (d1) in water; and
 - (d3) eluting adsorbed isoflavones from the washed resin of step (d2) with aqueous alcohol.
6. The process of claim 5 wherein step (d3) is followed by the further step of:
 - (d4) drying the eluted aqueous alcohol of step (d3) to reduce the isoflavones to a solid basis.
7. The process of claim 5 and the further step of evaporating the aqueous alcohol elution of step (d3) to reduce the isoflavones to a solid basis.
8. The process of claim 5 wherein the resin of step (d1) is selected from a group consisting of ionic ethylvinylbenzene-divinylbenzene, non-ionic ethylvinylbenzene-divinylbenzene, ionic styrene-divinylbenzene, non-ionic styrene-divinylbenzene, ionic polystyrene polymers, and non-ionic polystyrene polymers; and the aqueous alcohol of step (d3) is selected from a group consisting of ethanol, methanol, and isopropanol.
9. The process of claim 5 wherein the aqueous alcohol of step (d3) is ethanol.
10. The process of claim 2 wherein the constant temperature of step (a) is in the order of about 65-95°C.
11. The process of claim 10 wherein said ultrafiltration membrane of step (b) has a nominal molecular weight cut-off range of about 600-100,000.
12. The process of claim 10 wherein said ultrafiltration membrane of step (b) has a nominal molecular weight cut-off range of 10,000 nominal molecular weight.
13. The process of claim 11 wherein the back pressure of step (c) provides a permeate flow in the order of 70 mL/minute.
14. The process of claim 1 comprising the further step of adding water to the retentate of step (b) at a flow rate which is the same as the permeate rate or flux of the permeate, and the step of again passing the permeate with the added water through an ultrafilter.
15. A process of claim 2 comprising the added steps of:
 - (e) packing a liquid-chromatography column with a slurry of ethanol with a polymeric resin;
 - (f) back flushing the column with water until said resin expands by approximately fifty percent;
 - (g) feeding the permeate of step (b) through said column;
 - (h) eluting isoflavones from said resin by a use of ethanol; and
 - (i) drying the isoflavones eluted in step (h).
16. The process of claim 15 wherein said slurry of step (e) is about 50-100% wt ethanol followed by about 1-5 column volumes of 0.1% wt NaOH and 1-5 column volumes of water.
17. The process of claim 16 wherein the temperature of the permeate during step (g) is about 45-95°C.
18. The process of claim 15 and a further step after step (g) of washing said resin with water to remove residual sugars and other impurities therefrom.
19. The process of claim 15 wherein said step (i) is a drying step.
20. The process of claim 15 wherein said step (i) is a vacuum drying step.
21. A process for separating isoflavones in an aqueous vegetable starting material, said process comprising the steps of:

- (a) heating a vegetable starting material to an initial constant temperature in the order of about 65-95°C;
 (b) passing vegetable solubles produced in step (a) through an ultrafiltration membrane to provide a permeate;
 (c) gradually cooling the ultrafiltration permeate of step (b) from said initial hot temperature of about 85°C to a cooled temperature of about 22°C while constantly stirring said permeate,
 5 (d) centrifuging the cooled permeate of step (c) at about 900 x g for a period which is long enough to pelletize an isoflavones precipitate produced in step (d);
 (e) pouring off a supernatant;
 (f) diluting the precipitate with water;
 (g) recentrifuging the diluted precipitate of step (f) at about 900 x g to remove a residual supernatant; and
 10 (h) drying the recentrifugated precipitate of step (g).

22. The process of claim 21 wherein said vegetable starting material is soy molasses.

15 23. The process of claim 22 and the further steps of adding ethanol to the washed precipitate of step (f) while refluxing until the precipitate is dissolved, filtering the refluxed precipitate through a filter; and slowly cooling the filtered precipitate until fine crystals are formed.

20 24. The process of claim 23 and the added step of centrifuging said cooled and filtered precipitate to harvest the fine crystals, then pouring off the resulting supernatant, and finishing the process with said steps (g) and (h).

25 25. A process comprising the steps of separating insoluble solids from isoflavones in a soy molasses starting material by using ultrafiltration, a permeate stream resulting from said ultrafiltration containing an isoflavone profile corresponding to a profile of soy in the soy molasses starting material, and controlling a temperature throughout said process to select an isoflavone from said profile in order to separate and recover said selected isoflavone from said starting material.

30 26. A process comprising the steps of ultrafiltering a feed stream of soy molasses starting material to isolate isoflavones in an ultrafiltration permeate, recovering the isoflavones by adsorption of the isoflavones on a resin, washing said resin with water in order to remove residual impurities from the adsorbed isoflavones, and eluting the washed isoflavones with aqueous alcohol.

35 27. A process for recovering isoflavones from a starting material feed stream of soy molasses, said process comprising the steps of: heating said feed stream to a temperature which solubilizes genistin; ultrafiltering said heated feed stream to separate a purified genistin product in a permeate of said starting material, cooling said permeate of said filtered feed stream to promote genistin crystallization, and separating genistin crystals by a centrifugation or filtration of said cooled permeate.

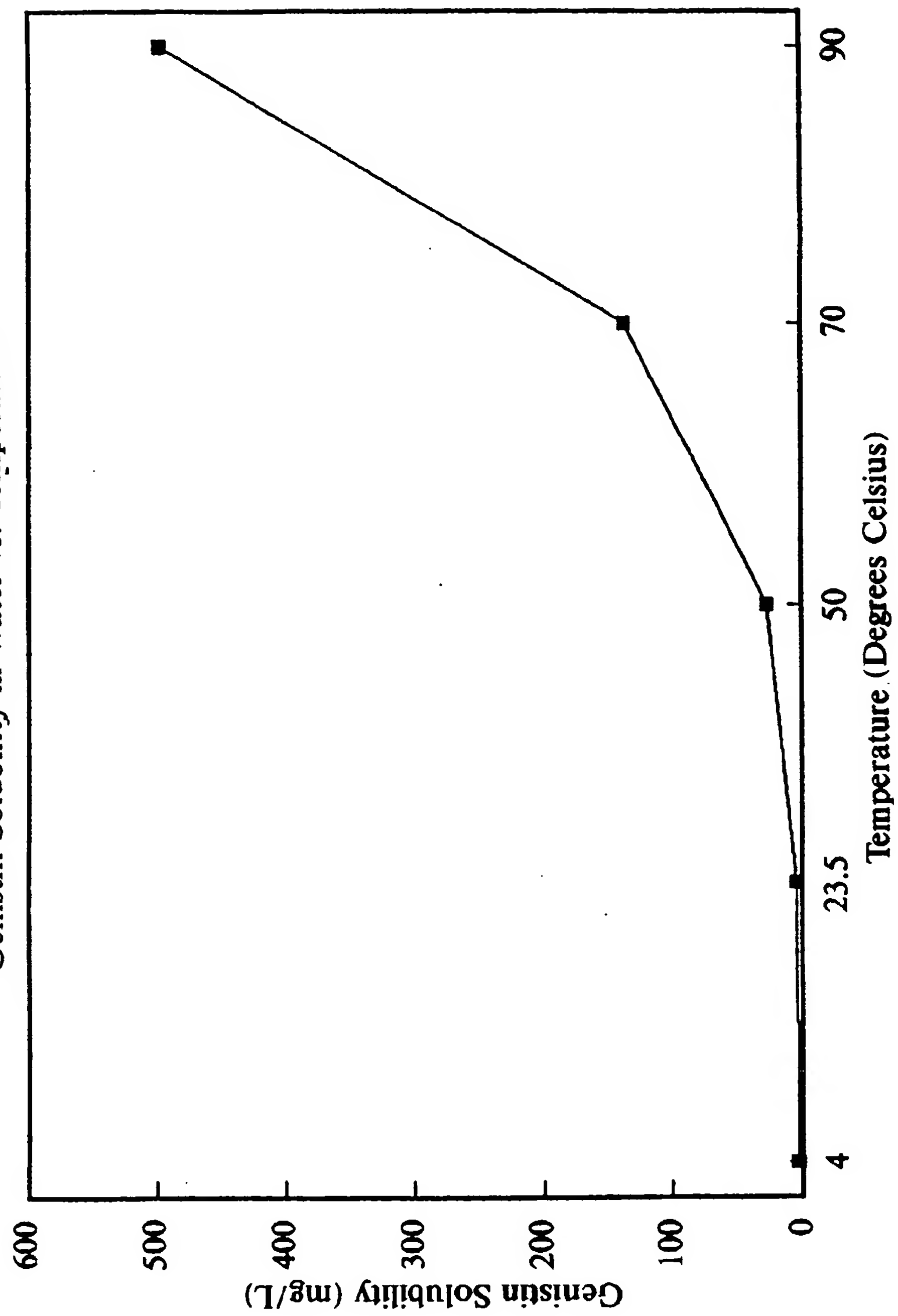
40 28. The process of claim 27 comprising the further steps of recrystallizing the genistin crystallization by forming a genistin precipitate from said crystallization of the ultrafiltration permeate, then making a heated aqueous alcohol solution containing said precipitate, and next recrystallizing the precipitate from a heated aqueous alcohol solution by allowing the solution to cool.

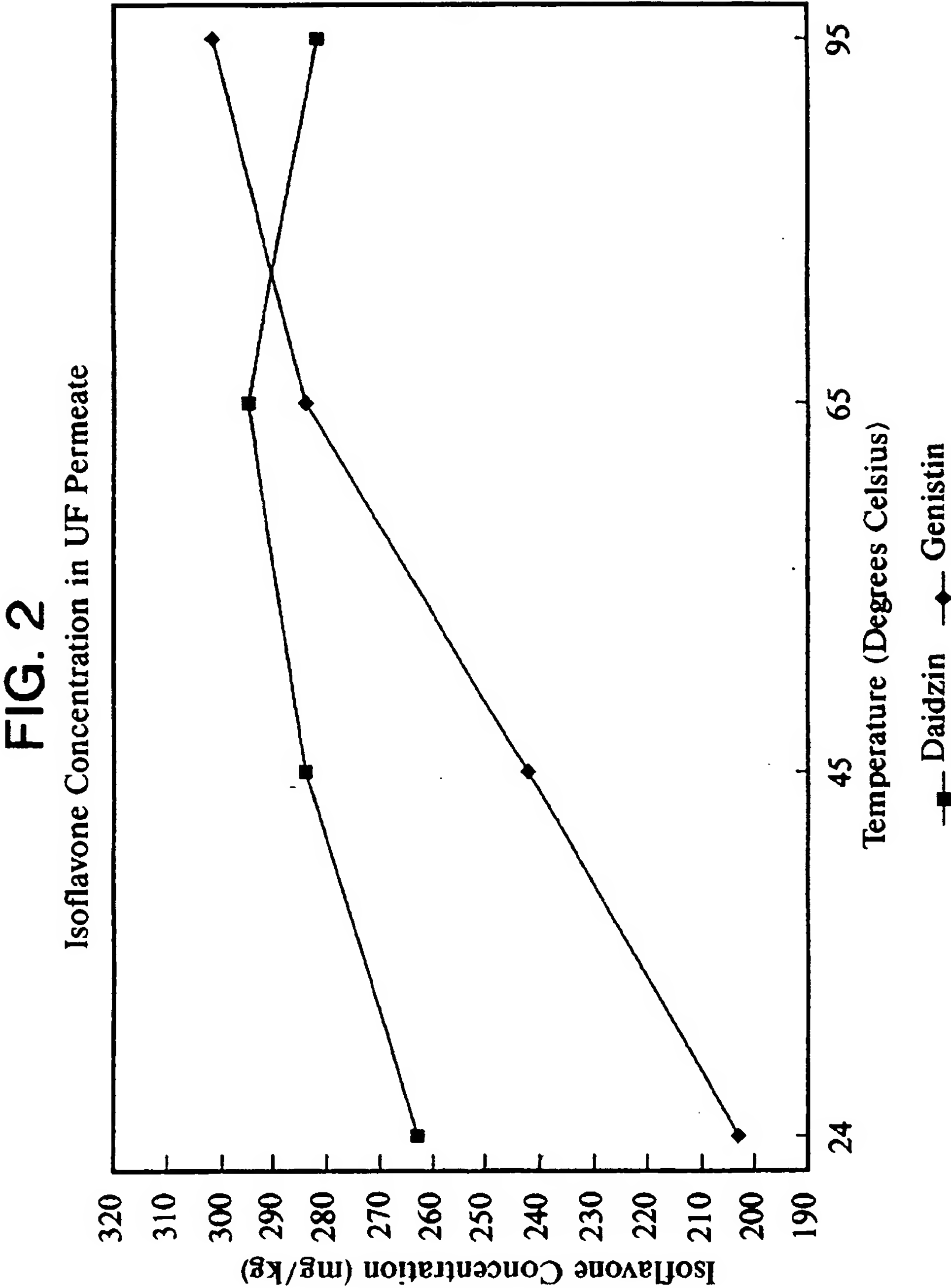
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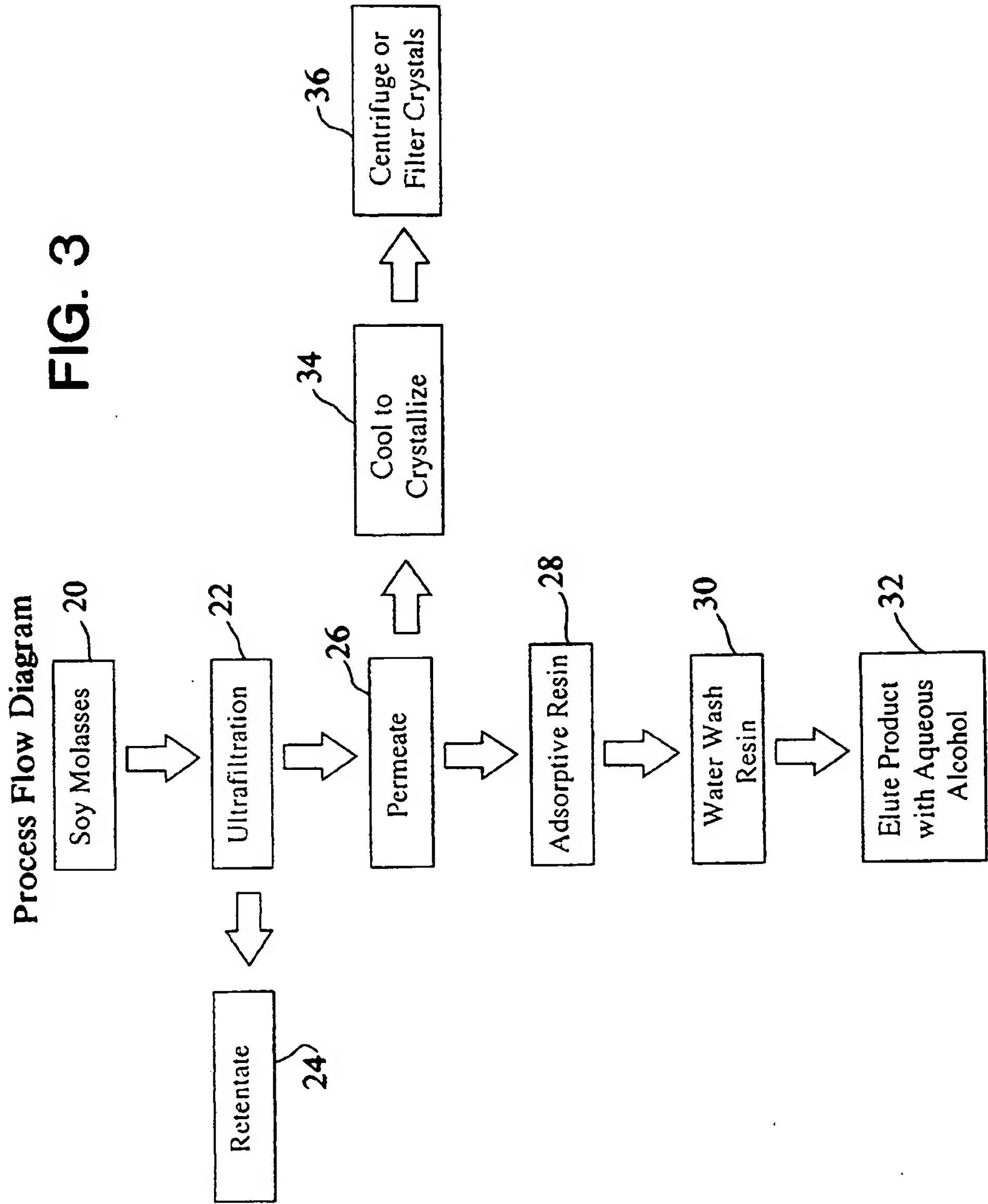
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FIG. 1
Genistin Solubility in Water vs. Temperature









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EUROPEAN SEARCH REPORT

Application Number
EP 97 30 1595

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	DATABASE WPI Week 9247 Derwent Publications Ltd., London, GB; AN 92-385398 XP002031911 & JP 04 283 518 A (KIKKOMAN CORP.) , 8 October 1992 * abstract *	1	C07D311/36
X	--- PATENT ABSTRACTS OF JAPAN vol. 096, no. 003, 29 March 1996 & JP 07 304655 A (KIKKOMAN CORP.), 21 November 1995, * abstract *	1	
Y,D	--- WO 95 10512 A (PROTEIN TECH INT ;SHEN JEROME L (US); BRYAN BARBARA A (US)) 20 April 1995 * claims 11,30,49 *	1	
Y	--- DATABASE WPI Week 8626 Derwent Publications Ltd., London, GB; AN 86-166658 XP002031912 & JP 61 100 524 A (JUNICHI IWAMURA K.K.) , 19 May 1986 * abstract *	1-27	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
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The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
BERLIN		4 June 1997	Frelon, D
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons --- & : member of the same patent family, corresponding document</p>			

EPI1 FORM 1503 01.92 (PUB/CI)



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EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	EP 0 348 781 A (TECNOFARMACI SPA ;INDENA SPA (IT)) 3 January 1990 * claim 9 *	1-27	
A	US 5 032 580 A (WATANABE KAZUHIRO ET AL) 16 July 1991 * column 3 *	1-27	
A	WO 95 03816 A (SCHWABE WILLMAR GMBH & CO ;CHATTERJEE SHYAM SUNDER (DE); JAGGY HER) 9 February 1995 * claims 1-4 *	1-27	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Place of search BERLIN		Date of completion of the search 4 June 1997	Examiner Frelon, D
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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